



## Final Scientific Report

### Cover Page

**BARD Project Number:** IS-4409-11 R

**Date of Submission of the report:** November 1, 2014

**Project Title:** Fine mapping, functional analysis and pyramiding of genes controlling begomovirus resistance in tomato.

#### Investigators

**Principal Investigator (PI):** Ilan Levin

**Co-Principal Investigator (Co-PI):** John W. Scott

**Collaborating Investigators:** Moshe Lapidot

Moshe Reuveni

#### Institutions

Volcani Center, ARO

Florida, U of

Volcani Center, ARO

Volcani Center, ARO

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**Keywords.** Pelota, DFDGD-class RNA-dependent RNA polymerase.

**Abbreviations.** Deoxyribonucleic acid (**DNA**); Quantitative Trait Loci (**QTL**); DFDGD-class RNA-dependent RNA polymerase(**DFDGD- RDRP**); *Tomato mottle virus* (**ToMoV**); *Tomato yellow leaf curl Virus* (**TYLCV**)

**Budget:** IS: 160,000 \$

US: 160,000 \$

**Total:** 320,000 \$

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Signature  
Principal Investigator

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Signature  
Authorizing Official, Principal Institution



## Final Scientific Report

### Publication Summary (numbers)

	Joint IS/US authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted) BARD support acknowledged		1		1
Submitted, in review, in preparation	1		1	2
Invited review papers				
Book chapters				
Books				
Master theses			1	1
Ph.D. theses				
Abstracts	2	1	1	3
Not refereed (proceedings, reports, etc.)	1			1

**Postdoctoral Training:** List the names and social security/identity numbers of all postdocs who received more than 50% of their funding by the grant.

### Cooperation Summary (numbers)

	From US to Israel	From Israel to US	Together, elsewhere	Total
Short Visits & Meetings	1	1	1	3
Longer Visits (Sabbaticals)				

**Description Cooperation:** The groups exchanged DNA marker information, seeds and biological knowhow that allowed substantial progress in the discovery of new genes that control TYLCV resistance and their utility.

### Patent Summary (numbers)

	Israeli inventor only	US inventor only	Joint IS/US inventors	Total
Submitted	1	1		2
Issued (allowed)				
Licensed				



## Final Scientific Report

**Abstract.** *Tomato yellow leaf curl virus* (TYLCV), a monopartite begomovirus, is one of the most devastating viruses of cultivated tomatoes and poses increasing threat to tomato production worldwide. Because all accessions of the cultivated tomato are susceptible to these viruses, wild tomato species have become a valuable resource of resistance genes. QTL controlling resistance to TYLCV and other begomoviruses (*Ty* loci) were introgressed from several wild tomato species and mapped to the tomato genome. Additionally, a non-isogenic F<sub>1</sub> diallel study demonstrated that several of these resistance sources may interact with each other, and in some cases generate hybrid plants displaying lower symptoms and higher fruit yield compared to their parental lines, while their respective resistance genes are not necessarily allelic. This suggests that pyramiding genes originating from different resistance sources can be effective in obtaining lines and cultivars which are highly resistant to begomoviruses. Molecular tools needed to test this hypothesis have been developed by our labs and can thus significantly improve our understanding of the mechanisms of begomovirus resistance and how to efficiently exploit them to develop wider and more durable resistance.

Five non-allelic *Ty* loci with relatively major effects have been mapped to the tomato genome using molecular DNA markers, thereby establishing tools for efficient marker assisted selection, pyramiding of multiple genes, and map based gene cloning: *Ty-1*, *Ty-2*, *Ty-3*, *Ty-4*, and *ty-5*. This research focused on *Ty-3* and *Ty-4* due to their broad range of resistance to different begomoviruses, including ToMoV, and on *ty-5* due to its exceptionally high level of resistance to TYLCV and other begomoviruses.

Our aims were: (1) clone *Ty-3*, and fine map *Ty-4* and *Ty-5* genes, (2) introgress each gene into two backgrounds and develop semi isogenic lines harboring all possible combinations of the three genes while minimizing linkage-drag, (3) test the resulting lines, and F<sub>1</sub> hybrids made with them, for symptom severity and yield components, and (4) identify and functionally characterize candidate genes that map to chromosomal segments which harbor the resistance loci.

During the course of this research we have: (1) found that the allelic *Ty-1* and *Ty-3* represent two alternative alleles of the gene coding DFDGD-RDRP; (2) found that *ty-5* is highly likely encoded by the messenger RNA surveillance factor PELOTA (validation is at progress with positive results); (3) continued the map-based cloning of *Ty-4*; (4) generated all possible gene combinations among *Ty-1*, *Ty-3* and *ty-5*, including their F<sub>1</sub> counterparts, and tested them for TYLCV and ToMoV resistance; (5) found that the symptomless line TY172, carrying *ty-5*, also carries a novel allele of *Ty-1* (termed *Ty-1<sup>V</sup>*).

The main scientific and agricultural implications of this research are as follows: (1) We have developed recombination free DNA markers that will substantially facilitate the introgression of *Ty-1*, *Ty-3* and *ty-5* as well as their combinations; (2) We have identified the genes controlling TYLCV resistance at the *Ty-1/Ty-3* and *ty-5* loci, thus enabling an in-depth analyses of the mechanisms that facilitate begomovirus resistance; (3) Pyramiding of *Ty* resistance loci is highly effective in providing significantly higher TYLCV resistance.



## Final Scientific Report

### *Achievements.*

**Significance of main scientific achievements or innovations:** During the course of this research we have cloned the gene controlling TYLCV resistance at the *Ty-1/Ty-3* locus. In addition we also, most probably, identified the gene controlling TYLCV resistance at the *ty-5* locus (validation is at progress with positive results). Finally, we have fine-mapped the gene controlling the virus resistance at the *Ty-4* locus.

Using the markers developed for the three loci mentioned we have introgressed the genes into two backgrounds, including their F<sub>1</sub> counterparts, and developed semi isogenic lines harboring all possible combinations of the three genes while minimizing linkage-drag. The resulting lines, and F<sub>1</sub> hybrids made with them, were tested for symptom severity and yield components. Our results show that in order to develop highly resistant cultivars there is a need to introgress at least two resistant genes, ideally in an homozygous state.

**Agricultural and/or economic impacts of the research findings:** : During the course of this research we have developed recombination free DNA markers that will substantially facilitate the introgression of *Ty-1*, *Ty-3* and *ty-5* as well as their combinations; We have identified the genes controlling TYLCV resistance at the *Ty-1/Ty-3* and *ty-5* loci, thus enabling an in-depth analyses of the mechanisms that facilitate TYLCV resistance; Finally: we have shown that pyramiding of *Ty* resistance loci is highly effective in providing significantly higher TYLCV resistance.

**Details of cooperation.** The Israeli and the US group exchanged marker information on a continuous basis; The Israeli team has sent seeds of TY172 to the US. Seeds of plants carrying *Ty-3* and *Ty-4* were sent by the US group to Israel; Crosses among genotypes were carried out in both countries but due to a delay in Israel, the US group sent seeds of advanced backcrosses of the two backgrounds to Israel. The Israeli team has inoculated the resulting plants with TYLCV; The US group has inoculated plants with ToMoV, a virus that does not exist in Israel; The two groups have presented collaborative data in the International *Symposium on Tomato Diseases, Orlando, Florida, USA*; The American collaborator, Jay Scott visited Israel to observe ongoing studies; The Israeli PI visited Florida to observe ongoing studies; The American collaborators (Scott and Hutton) and two Israeli collaborators



## Final Scientific Report

(Levin and Lapidot) participated in the former *Tomato Breeders Round Table meeting*, which took place at *Chiang Mai, Thailand* at February 2012. Both groups presented their results, and the four collaborators discussed the progress of the BARD project; The American collaborators (Scott and Hutton) presented BARD research data during the last *Tomato Breeders Round Table meeting*, which took place at *Mountain Horticultural Crops Research and Extension Center (MHCREC), Mills River, NC, USA* on September 2014; The American collaborators (Scott and Hutton) and two Israeli collaborators (Levin and Lapidot) submitted a manuscript to *Molecular breeding*, currently under review.

### ***List of Publications.***

Verlaan MG, Hutton SF, Ibrahem RM, Kormelink R, Visser RG, Scott JW, Edwards JD, Bai Y. 2013. The Tomato Yellow Leaf Curl Virus resistance genes *Ty-1* and *Ty-3* are allelic and code for DFDGD-class RNA-dependent RNA polymerases. *PLoS Genet.* 9(3):e1003399. doi: 10.1371/journal.pgen.1003399.